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EXAMINER	
CANELLA, KAREN A	
ART UNIT	**PAPER NUMBER**
1642	

DATE MAILED: 04/20/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/033,577	VALLERA ET AL.	
	Examiner	Art Unit	
	Karen A Canella	1642	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☐ Claim(s) *1-5,7-20,25,26 and 30-32* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☐ Claim(s) *1-5, 7-20, 25, 26 and 30-32* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Claims 6, 21-24 and 27-29 have been canceled. Claims 30-32 have been added. Claims 1-5, 7-20, 25, 26 and 30-32 are pending and under consideration.

Text of sections of Title 35, US code, not found in this action can be found in a previous action.

The rejection of claim 26 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention is maintained for the following reason of record. It is unclear how the limitation of "an article of manufacture" would further limit the fusion toxin of claim 21 or the pharmaceutical composition of claim 25..

Claims 1-5 and 7-20, 25, 26 and 30-32 are rejected under 35 U.S.C. 103(a) as being unpatentable over Rajagopal et al (Journal of Biological Chemistry, 2000, vol. 275, pp. 7566-7573, cited in a previous Office action) or and Oldfield et al (Current Topics in Microbiology and Immunology, 1998, Vol. 234, pp. 97-114, cited in a previous Office action) both in view of Greenfield et al (Science, 1987, vol. 238, pp. 536-539, cited in a previous Office action) and Fabbrini et al (FASEB Journal, 1997, Vol. 11, pp. 1169-1176, cited in a previous Office action) and Mori et al (Journal of Neuro-Oncology, 2000, Vol. 46, pp. 115-123, cited in a previous Office action).

Claim 1 is drawn to a method for killing a tumor cell comprising contacting said tumor cell with a fusion toxin comprising the toxin domain of diphtheria toxin and a urokinase-type plasminogen activator domain, wherein said contacting is done in vivo Claim 2 embodies the method of claim 1 herein said tumor cell s a brain tumor cell. Claims 3 and 4 embody the method of claim 2 wherein said brain tumor is selected from the group consisting of glioblastoma. Because of the Requirement of the election of species in Paper No. 5, claims 1-4 will be examined to the extent that they read on glioblastoma. Claim 5 is drawn to the method of claim 1 wherein said tumor cell expresses the urokinase-type plasminogen activator receptor. Claim 7 embodies the method of claim 1 wherein said fusion toxin comprises the translocation enhancer region of diphtheria toxin. Claim 8 embodies the method of claim 1 wherein said fusion toxin comprises the amino terminal 390 amino acids of diphtheria toxin. Claim 9 is

drawn to the method of claim 1 wherein said urokinase-type plasminogen activator domain is capable of binding to urokinase-type plasminogen activator receptor. Claim 10 embodies the method of claim 9 wherein said plasminogen activator domain comprises the amino terminal fragment of urokinase type plasminogen activator. Claim 11 embodies the method of claim 1 wherein said fusion toxin comprises the toxin domain of diphtheria toxin, the translocation enhancing region of diphtheria toxin and the amino terminal fragment of urokinase type plasminogen activator.

Claim 12 is drawn to a method for killing a glioblastoma tumor cell comprising contacting said tumor cell with a fusion toxin comprising a urokinase-type plasminogen activator domain, wherein said contacting occurs in vivo. Claim 13 is drawn in part to the method of claim 12 wherein said fusion toxin comprises a toxin domain of diphtheria toxin. Claim 14 embodies the method of claim 12 wherein said fusion toxin comprises the toxin domain of diphtheria toxin. Claim 15 is drawn in part to the method of claim 12 wherein said fusion toxin comprises an internalization domain of diphtheria toxin. Claim 16 embodies the method of claim 12 wherein said fusion toxin comprises the translocation enhancing region of diphtheria toxin. Claim 20 embodies the method of claim 12 wherein said fusion toxin comprises the toxin domain of diphtheria toxin, the translocation enhancing region of diphtheria toxin, and the amino terminal fragment of the urokinase-type plasminogen activator.

Claim 25 is drawn to a pharmaceutical composition comprising a fusion toxin, wherein said fusion toxin comprises the toxin domain of diphtheria toxin and a urokinase-type plasminogen activator. Claim 25 is drawn to an article of manufacture comprising the pharmaceutical composition of claim 25. Claim 30 embodies the fusion toxin of claim 25 wherein said fusion toxin further comprises the translocation enhancing region of diphtheria toxin. Claim 31 embodies the fusion toxin of claim 25 wherein said urokinase-type plasminogen activator domain comprises the amino terminal fragment of urokinase-type plasminogen activator. Claim 32 embodies the fusion toxin of claim 25 wherein said exotoxin comprises the toxin domain of diphtheria toxin, the translocation enhancing region of diphtheria toxin and the amino terminal fragment of urokinase-type plasminogen activator.

Rajagopal et al teach a method of killing a glioblastoma cell comprising administering a fusion toxin comprising the internalization domains and toxin domains of Pseudomonas exotoxin

and an amino terminal fragment of urokinase plasminogen activator, for the reasons set forth above. Rajagopal et al teach that the representative number of urokinase plasminogen receptors on glioblastoma cell lines range from 670 sites/cell to 106 sites/cell (page 7570, Table II). Rajagopal et al do not teach a method for killing glioblastoma cells comprising administering fusion toxins comprising the toxin or internalization domains of diphtheria toxin.

Oldfield et al teach a method of treating a patient having glioblastoma comprising administering a fusion toxin wherein said fusion toxin comprises a mutant diphtheria toxin as taught by Greenfield et al fused to transferrin. Oldfield et al do not teach a method for treating a patient having glioblastoma comprising administering a fusion toxin wherein said fusion toxin comprises a urokinase plasminogen activator.

Fabbrini et al teach a method of killing cells expressing the urokinase plasminogen activator receptor comprising contacting said cells with a fusion toxin comprising the amino terminal fragment of urokinase plasminogen activator and the toxin domain of saporin (page 1173, under the heading "Cell killing experiments") Fabbrini et al teach that pro-urokinase was able to inhibit the cytotoxicity of the recombinant saporin fusion protein thus demonstrating that the cytotoxicity of said fusion protein is due to binding to the urokinase plasminogen activator receptor (page 1173, lines 1-8 under the heading "Competition Experiments"). Fabbrini et al teach that the amino terminal fragment of urokinase plasminogen activator is necessary for targeting the receptor of urokinase plasminogen activator, but that internalization at said receptor takes place because of the toxin (page 1175, column 1, lines 1-5, first full paragraph).

Greenfield et al teach recombinant mutant forms of diphtheria toxin which retain membrane translocation ability but loose the ability to bind to the diphtheria toxin receptor. Greenfield et al that immunotoxins comprising said mutant diphtheria toxins are much more effective in killing target cells than immunotoxins comprising only the toxin domain of diphtheria toxin. Therefore one of skill in the art would conclude that it is advantageous to include the membrane translocation domain with the toxin domain in a fusion protein to be targeted to a specific cells type.

Mori et al teach that urokinase-type plasminogen activator receptor is unregulated in glioma cells exhibiting enhanced invasion activity such as in glioblastoma multiforme (abstract).

It would have been prima facie obvious to one of ordinary skill in the art at the time the claimed invention was made to administer to a patients having glioblastoma a fusion protein comprising a mutant diphtheria toxin lacking the ability to bind to the diphtheria toxin receptor, but maintaining the translocation and toxin domains and the amino terminal fragment of urokinase plasminogen activator. One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of Fabbrini et al on the toxin mediated cytotoxicity of fusion toxins comprising the amino terminal fragment of urokinase plasminogen activator and saporin and the teaching of Greenfield et al on the mutant diphtheria toxins which are lacking diphtheria toxin receptor binding activity by maintaining the ability to translocate into the cytosol. One of skill in the art would recognize that such a fusion toxin comprising diphtheria toxin would also be effective in a method of killing glioblastoma cells. It would have been prima facie obvious to one of ordinary skill in the art at the time the claimed invention was made to substitute the amino terminal fragment of urokinase plasminogen activator for transferrin in the method of treating as taught by Oldfield et al. One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of Rajagopal et al on the representative number of urokinase plasminogen receptors on glioblastoma cell lines which range from 670 sites/cell to 106 sites/cell (page 7570, Table II) and the teachings of Mori et al on the up regulation of urokinase type plasminogen activator receptor in glioblastoma multiforme. Thus, one of skill in the art would be motivated to target the plasminogen activator receptor because glioblastoma cells would be expected to expresses numerous receptor molecules

Claims 13 and 15 are rejected in further view of Leppla et al (U.S. 5,591,631, cited in a previous Office action) and Wels et al (WO 96/13599, cited in a previous Office action) and Arnon (WO 94/26308, cited in a previous Office action) and McDonald et al (WO 00/04926, cited in a previous Office action) and Morishita et al (Nucleic Acids symposium series, 1996, Vol. 35, pp. 291-292, cited in a previous Office action) and Pastan et al (Journal of Biological Chemistry, 1989, Vol. 264, pp. 15157-15160, cited in a previous Office action) and Baty et al (Molecular Microbiology, 1988, Vol. 2, pp. 807-811, cited in a previous Office action) and el Kouhen et al (European Journal of Biochemistry, 1993, vol. 214, pp. 635-639, cited in a previous Office

action) and Geoff et al (Protein Engineering, 1997, Vol. 10, suppl. page 5, cited in a previous Office action) and Bouveret et al (Molecular Microbiology, 1997, vol. 23, pp. 909-920, cited in a previous Office action) and Lacy et al (Journal of Molecular Biology, 1999, Vol. 291, pp. 1091-1104, cited in a previous Office action) and Wiedlocha et al (Cancer Research, 1991, Vol. 51, pp. 916-920, cited in a previous Office action) and Olsnes et al (Journal of Biological Chemistry, 1982, Vol. 257, pp. 13263-13270, cited in a previous Office action).

Claim 13 is further drawn to the method of claim 12 wherein said fusion toxin comprises a toxin domain of a toxin selected from the group consisting of colicin, anthrax toxin, tetanus toxin, botulinum toxin, saporin, abrin, bryodin, pokeweed anti-viral protein, viscumin and gelonin. Claim 15 is further drawn to the method of claim 12 wherein said fusion toxin comprises an internalization domain of a toxin selected from the group consisting of colicin, delta-endotoxin, anthrax toxin, tetanus toxin and botulinum toxin.

The combination of prior art references render obvious claims 13 and 15 to the extent that they read on the toxin domain of diphtheria toxin and the internalization domain of diphtheria toxin, respectively. Fabbrini et al teach a method of killing cells expressing the urokinase plasminogen activator receptor comprising contacting said cells with a fusion toxin comprising the amino terminal fragment of urokinase plasminogen activator and the toxin domain of saporin (page 1173, under the heading "Cell killing experiments") Fabbrini et al teach that pro-urokinase was able to inhibit the cytotoxicity of the recombinant saporin fusion protein thus demonstrating that the cytotoxicity of said fusion protein is due to binding to the urokinase plasminogen activator receptor (page 1173, lines 1-8 under the heading "Competition Experiments"). Fabbrini et al teach that the amino terminal fragment of urokinase plasminogen activator is necessary for targeting the receptor of urokinase plasminogen activator, but that internalization at said receptor takes place because of the toxin (page 1175, column 1, lines 1-5, first full paragraph). Thus the combination of prior art references renders obvious the method of claim 12 wherein said toxin domain is saporin in addition to diphtheria toxin. The combination of prior art references do not teach a method for killing glioblastoma cells comprising administering fusion toxins comprising the toxin domains of colicin, anthrax, tetanus, botulinum, abrin, bryodin, poke-weed anti-viral protein, viscumin or gelonin, or an internalization domain of colicin, delta-endotoxin. Anthrax, tetanus, or botulinum.

Leppla et al (U.S. 5,591,631) teach anthrax-toxin fusion proteins and hybrid toxins and immunotoxins comprising anthrax toxin lethal factor fused to the toxin domain of Pseudomonas exotoxin. Leppla et al identify the anthrax toxin lethal factor as the translocation domain of anthrax toxin. One of skill in the art would conclude that the translocation domains are functional outside of the context of the wild type toxin as Leppla et al teach that the hybrid toxin comprising P exotoxin and the anthrax lethal factor increases the toxicity of the P exotoxin by means of increasing translocation of the P exotoxin into the cytosol

Arnon teaches immunotoxins comprising the toxin domains of botulinum or tetanus toxins.

McDonald et al teach immunotoxins comprising gelonin, saporin, abrin, ricin, colicin and pokeweed anti-viral protein.

Wiedocha et al teach an immunotoxin comprising the toxin domain of mistletoe lectin I. Olsnes et al teach that viscumin is a toxic lectin from mistletoe.

Baty et al and el Kouhen et al and Geoff et al and Bouveret et al teach the translocation domains of colicin A, colicin N, colicin E9, and colicin E3, respectively.

Lacy et al teach the translocation domains of botulinum and tetanus neurotoxins.

Pastan et al teach the translocation domain of Pseudomonas exotoxin.

Wels et al teach the translocation domains of colicin A, and d-endotoxin.

Morishita et al teach the translocation domain of ricin.

It would have been prima facie obvious to one of ordinary skill in the art at the time the claimed invention was made to substitute the toxin domain of colicin, anthrax, tetanus, botulinum, saporin, abrin, bryodin, pokeweed anti-viral protein, vescumin or gelonin for the toxin domain of Pseudomonas exotoxin in the method of killing glioblastoma cells as taught by Rajagopal et al; it would also be obvious to substitute the translocation domain of colicin, delta-endotoxin, anthrax, botulinum, or tetanus for the translocation domain of P endotoxin in the method of killing glioblastoma cells as taught by Rajagopal et al. One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success

a. by the teachings of Arnon and McDonald et al and Wiedocha et al which identify the toxic domains of botulinum, tetanus, gelonin, saporin, abrin, ricin, colicin, pokeweed anti-viral protein and viscumin;

b. by the teachings of Baty et al and el Kouhen et al and Geoff et al and Bouveret et al and Lacy et al and Pastan et al and Wels et al and Morishita et al which identify the translocation domains for colicins, botulinum, tetanus, P endotoxin and ricin;
c. by the teachings of Fabbrini et al on the contribution of the toxin moiety toward internalization at the urokinase plasminogen activator receptor and the teachings of Rajagopal et al on the importance of facilitating transportation of the toxin domain into the cytosol; and
d. by the teachings of Leppala on the independent functioning of the translocation domain and the toxin domain.
One of skill in the art would recognize by the teachings Leppala et al that the fusion toxin can be effective with any toxin which has be utilized in the art as an immunotoxin and that the translocation domain is synonymous for the internalization domain and that any of the aforesaid translocation domains can be fused to any of the aforesaid toxin domain in a fusion toxin comprising urokinase plasminogen activator to facilitate delivery of a toxin into the cytoplasm of the glioblastoma tumor cell expressing the urokinase plasminogen activator receptor.

Applicant argues against the prior art reference stating that there is lack of a reasonable expectation of success due to the potential for non-target toxicity which is commonly recognized in the art. applicant further argues that one of skill in the art would appreciate that since the brain has low threshold for bleeding, administration of an anti-vascular fusion toxin to the brain could result in vessel damage. Applicant argues that that a dose limiting side effect is vascular leak syndrome applicant points of the work of Hagihara et al who reported neurological deficits consistent with endothelial damage following the interstitial microinfusion of transferrin-diphtheria toxin into brain tumor patients. Applicant cites Baluna et al and Hagihara et al and Merril et al and Hall in support for this position. This has been considered but not found persuasive. Baluna et al deduced that a VDL-related peptide binding motif with endothelial integrin receptor binding function was necessary for toxin-induced VLS, and hypothesize that fusion proteins lacking these sequences would cause less VLS. Hagihara et al teach that cortical necrosis associated with the administration of transferrin-CRM107 are associated with higher doses of said fusion protein, and the injury to normal brain can be blocked by prophylactic administration of chlorquine. Further, Oldfield et al teach that the sensitivity of normal brain to

anti-transferrin receptor immunotoxins is about 3000 times lower than the sensitivity of numerous glioma cell lines (page 99, lines 5-7), suggesting that a therapeutic window which would allow a proper dose to be administered to treat glioma cells and not intoxicate normal brain cells would be possible. Olfield et al teach that receptor-specific uptake is much more efficient than non-specific uptake and therefore doses of immunotoxins which are too high will result in non-specific uptake rather than receptor-mediated uptake (page 103, lines 3-5). Olfield et al teach that neurological aberrations documented in monkeys after administration of fusion toxins occur at high concentrations several logs higher than those necessary for in vitro anti-tumor activity against many human tumors (page 103, lines 8-14). Rajagopal et al teach that normal brain does not express the receptor for uPA in contrast to glioblastoma cell lines which express up to 106 uPAR per cell (page 7573, first column, lines 16-19). Rajagopal et al teach although uPAR is expressed on normal liver cells prohibiting the systemic administration of fusion proteins comprising uPA, intratumoral administration of said fusion proteins would be advised (page 7573, lines 3-4 and lines 24-27). Oldfield et al also teaches the direct delivery of a fusion toxin to a tumor sites by means of intrathecal delivery (section 4.2). This is corroborated by the findings of Hall who teaches that direct intratheal administration of immunotoxins in animals yielded promising results (page 549, first column, first paragraph under the heading "Toxicity Studies"). Hall cites human clinical trials patients receiving intrathecal 454A12-RAresulted in CSF toxicity only for patients receiving doses of over 120 micrograms. Hall states that in patients receiving higher concentrations of transferrin receptor-CRM107 conjugates focal peritumoral brain injury was seen 2 to 4 weeks after treatment. Thus, upon reading of Olfield et al and Rajgopal et al one of skill in the art would ascertain that interthecal delivery of an optimized dose of a fusion toxin comprising uPA would exert a toxic effect on the glioblastoma cells expressing uPA, and would not cause widespread bleeding and necrosis within the brain, these effects being observed only when higher doses of fusion toxins were used. With regard to the teachings in the abstract of Merrill et al, it is noted that the fusion toxin used by Merrrill et al was specifically targeting tumor vasculature, rather than the tumor tissue itself, because VEGF receptors are upregulated on newly formed vasculature rather than on tumor tissue. This is in contrast to uPAR which is upregulated on the glioblastoma tumor tissue and not the tumor vasculature.

All other rejections and objections as set forth in the previous Office action are withdrawn.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Karen A Canella whose telephone number is (571)272-0828. The examiner can normally be reached on 10 a.m. to 9 p.m. M-F.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Yvonne Eyler can be reached on (571)272-0871. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

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PRIMARY EXAMINER